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THE OCCURRENCE AND DISTRIBUTION OF THE LACTIC STREPTOCOCCI IN EDMONTON MILK AND CREAM

G.A. Nelson

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1951
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THE OCCURRENCE AND DISTRIBUTION OF THE LACTIC STREPTOCOCCI IN EDMONTON MILK AND CREAM

A DISSERTATION
SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

FACULTY OF AGRICULTURE
DEPARTMENT OF DAIRYING

by
GORDON ALBERT NELSON, B.Sc.

EDMONTON, ALBERTA,

Thesis
1951
#35

University of Alberta

Faculty of Agriculture
Department of Dairying

The undersigned hereby certify that they have read and recommend to the School of Graduate Studies for acceptance, a thesis entitled:

THE OCCURRENCE AND DISTRIBUTION OF THE LACTIC
STREPTOCOCCI IN EDMONTON MILK AND CREAM,

submitted by Gordon Albert Nelson, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

PROFESSOR H.R. Thornton

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Date 10/4/51

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ABSTRACT

The lactic streptococci were found to occur normally in sweet raw milks collected from Edmonton dairy plants but the proportion of the total bacterial flora made up of these microorganisms was small. In addition there was a lower incidence of lactic streptococci in the winter than in the spring or summer milks. The fecal streptococci made up a much smaller proportion of the total bacterial flora of sweet raw milks than the lactic streptococci did. The lactic streptococci were found to be largely responsible for the souring of 15 churning creams, 15 of 18 sweet raw milks and 5 of 10 pasteurized milks but were not responsible for the souring of 3 aseptically drawn milks. When the lactic streptococci were not responsible for the souring of milks, the fecal streptococci or the acid formers (not lactic or fecal) were responsible.

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THE OCCURRENCE AND DISTRIBUTION
OF THE LACTIC STREPTOCOCCI
IN EDMONTON MILK AND CREAM

I. INTRODUCTION

The purpose of this investigation was primarily to find whether or not Edmonton's market milk supply is heavily seeded with the lactic streptococci. Also of importance was to find whether or not the lactic streptococci or the fecal streptococci (enterococci) are responsible for the souring of Edmonton's milks.

This knowledge is of economic importance since sour milk and cream cannot be used for many purposes for which the sweet products are and therefore are less valuable. On the other hand if the milks going into cheese factories were heavily seeded with the lactic streptococci, the initial production of lactic acid would be greater and a better quality of cheese would be assured.

II. HISTORICAL

Hammer(11) has stated that lactic acid fermentation occurs regularly in raw milk in all parts of the world and its rate is proportional to the temperature at which the milk is held.

Hammer(11) has further stated that milk leaving the stable practically always contains Streptococcus lactis, but the percentage of the total flora made up of this organism is small.

Evans(9), in studying the flora of milks freshly drawn

from the udders of cows, did not find any of the microorganisms to be *S. lactis*. Therefore, it would seem that contamination of raw milks with *S. lactis* comes from exterior sources and the distribution and numbers of these lactic streptococci is of importance. Ayers and Mudge(5) in 1922 reported they found no lactic streptococci in udder milks.

Holing(13) in 1904 isolated streptococci from the feces of infants, horses and other domestic animals , and was able to show that sterile skim milk coagulated typically when inoculated with these organisms.

Barthel(13) in 1906 found organisms which he called *S. lacticus*, known to us as *S. lactis*, widely distributed on meadows, plants, and cultivated land but scarce and in enfeebled condition on wooded land. In the dairy he found it in cow manure, in dust of air, in water, very commonly on the coat of the animal, on hay, straw, in oil cake, bran, malt and even on flies.

Esten(10) at the Connecticut Experiment Station in 1910 investigated the sources of organisms which he classed as *Bacterium lactis acidi*, known to us as *S. lactis*. Except in cases thought to be accidental, this species was not found in different soils or hay, on leaves of trees and plants, or on grass. Of the grains tested, i.e., cotton-seed meal, gluten bran, middlings, mixed feed and corn meal, only corn meal contained any of the cells of *S. lactis*. Several parts of the human body were tested but no positive result was obtained. However, the inside of the human mouth gave evidence of a large colonization. *S. lactis* was present in the feces of 11 of 25 cows, but these

cultures had but little power to curdle milk. Esten further found the organisms in abundance in the mouths and on the exteriors of nearly all of the cows and in the feed troughs and mangers. Eventually the organisms were found in the cow's intestine.

Allen(3), at Washington, identified as S. lactis 9.2% of 250 cultures of bacteria from fresh feces.

Ayers and Mudge(6) in 1923 with more modern methods of study, did not find S. lactis among the characteristic streptococci of the bovine mouth, throat or feces.

Stark and Sherman(22) referred to these earlier investigations and said that quite possibly these workers had failed to differentiate between S. lactis and S. fecalis. Stark and Sherman soaked several grains and vegetables in sterile distilled water and plated and incubated the infusion on milk agar. Similarly feces, soil and swabs of cow's throats were examined. They concluded that S. lactis is found on plants and suggested that this source is a probable one from which it finds its way into milk. Further, they did not find the organism in the mouths or the throats of cows, bovine feces, human feces or soil.

Perhaps the most common source of S. lactis on the farm is the dairy utensils. Whiting(23), at the New York Experiment Station, studied the bacterial flora of milk cans. Of the organisms isolated, 50% were able to produce undesirable fermentations in milk. This percentage did not include those producing a weak acid or alkaline reaction, the S. lactis, or the inert group. He found that the utensils did not introduce large numbers of S. lactis into milk and that these

microorganisms constituted only 3.9% of the total bacteria in cans. However, his temperature -medium combination was one which would not favor the lactic streptococci.

Since Stark and Sherman's work in 1935 other work has been done on the distribution of S. lactis and other bacteria in various dairy products.

It may be of interest - at least as far as the fecal streptococci are concerned - to mention that in 1940, a study of the weakly hemolytic streptococci of bovine mastitis by Slanetz and Naghski(21) showed that of 680 streptococcal cultures 84.2% were identified as S. agalactiae, 12.2% as S. uberis, 2.2% as S. dysgalactiae and 1.3% as S. fecalis.

Rowlands and Provan(19) in 1941 investigated the bacterial flora of milk pasteurized by the "in the bottle" process. Such milk was kept at 15.5°C. and examined at intervals. In freshly pasteurized samples the predominant bacteria were slow acid formers such as Microbacterium (Orla-Jensen) or Corynebacterium. After 48 hours alkali formers and peptonizers were predominant, while after 72 hours S. lactis made up the bulk of the flora and produced acid rapidly. Thermophilic bacteria died out rapidly at 15.5°C.

Pasteurized milk examined over a period of five years in Virginia showed that the type species of S. lactis was almost entirely replaced by the variant tardus(18).

In 1942 raw milk samples from 480 Omaha dairies were plated in beef infusion blood agar and per ml. counts ranging from 0 to 12,000 for hemolytic streptococci and 0 to 76,000 for non-hemolytic

streptococci were obtained(7). Of the non-hemolytic streptococci isolated, 71 cultures from 39 dairies were classified and included 3 cultures of S. salivarus, 9 of S. bovis and 14 of S. lactis and 13 of S. fecalis.

Abd-El-Malek and Gibson(2) in 1948 collected raw milk samples, some being aseptically drawn, others of good quality and some from bulked lots. Each sample was examined in the raw and pasteurized state, pasteurization being carried out at 145.5°F. for 30 minutes. Twenty-two of 23 of the raw milk samples yielded streptococci. Of these 22 samples, S. kefir was detected in 19, S. lactis in 17, mastitus streptococci in 16, S. cremoris in 10, S. fecalis in 7, S. citrovorus in 3, and S. bovis in 2. There were no cultures of S. lactis in the milk samples that were pasteurized.

Nichols and Hoyle at Reading(17), on isolating 72 cultures of lactic streptococci from sour milk suitable for cheesemaking, identified all strains, with two possible exceptions, as S. lactis.

It would seem from this accumulation of data that the lactic streptococci have as their habitat plants and dairy products and not bovine feces, bovine throats or soil as presupposed by the early investigators. Since lactic streptococci are not thermoduric, they would be more often found in raw than in heat-treated dairy products, unless introduced after heat treatment.

The chief concern of this investigation is to find what proportion of the bacterial flora of Edmonton raw milks are made up of the lactic streptococci and in addition to find what

streptococci are souring our Edmonton milks. No report of a quantitative nature could be found dealing with the proportion of lactic streptococci as compared to other bacteria present in milk. As has been mentioned, many workers estimated the percentage of lactic streptococci in the total number of isolations which would be at best only a semi-quantitative method.

Various media have been used in studying the lactic streptococci. Hammer of Iowa used various meat infusion media in his work with the lactics. Joshi and Ram Ayyar(16) working with an aroma producing lactic streptococcus used purple lactose agar, the indicator possibly being brom-cresol purple. Hunter(14) of New Zealand found that a yeast extract, beef extract, lactose, peptone and buffered agar was a superior medium for lactic streptococci. Later he(15) substituted tryptone for peptone and glucose for lactose in order to grow a variant of S. lactis.

III. PROCEDURES AND METHODS OF ANALYSIS

(a) Choice of a medium.

The first task was to find a suitable solid medium to support maximum growth of the lactic streptococci.

In preliminary trials the following media were used:-

(1) Veal infusion dehydrate (23 gms.), 10 gms. of glucose, 12 gms. of agar, 1,000 ml. of distilled water.

(2) The same as (1) with 10 gms. of tryptone added.

(3) Beef extract (1 gm.), 10 gms. of proteose peptone, 5 gms. of sodium chloride, 16 gms. of bacto-agar, 0.02 gms. of brom-cresol purple indicator, 10 gms. of glucose, 1,000 ml. of distilled water.

(4) T. G. E. M. agar (1) prepared according to Standard Methods for the Examination of Dairy Products.

(5) Hunter's first medium: 2% agar, 1% beef extract, 2% lactose, 1% peptone, 10% yeast extract and 0.6% disodium phosphate.

(6) Hunter's second medium in which he used tryptone instead of peptone and glucose instead of lactose.

(7) A modification of (2) above in which the solid materials were a little more concentrated: 23 gms. of veal infusion dehydrate, 1,000 ml. of distilled water, 12.7 gms. of agar, 10.6 gms. of glucose and 10.6 gms. of tryptone.

A raw milk sample obtained from one of the dairies in Edmonton was plated on the first six media ((1) to (6)) and colonies were fished from the plates into litmus milk. None of

the six media yielded any lactic streptococci. The three media giving the greatest number of acid formers were media (2), (3) and (6). These three media, when tried on a second raw milk sample, gave lactic streptococcus counts of 5,000 for medium (2), 2,000 for medium (3) and 2,000 for medium (6). All media were incubated at 25°C. for 4 days, since lactic streptococci have an optimum temperature range for growth of 20-30°C. The cultures believed to be lactic streptococci obtained from medium (2) were tested in litmus milk for growth at 45°C. and two of the cultures were found to be fecal streptococci. Nevertheless medium (2) still supported the growth of a higher percentage of lactic streptococci.

Two tubes of sterile skim milk were inoculated with a mixed strain starter called "Flavolac" and incubated at 25°C. until the skim milk went sour as determined by taste. On plating on six media, i.e. media (2) to (7), plate counts were determined of the sterile skim milk soured by this starter after being incubated at 25°C. for 4 days. The highest counts were obtained on media (2) and (4).

Media (2) and (4) were then experimented with in much the same manner as above except that, in addition to using the "Flavolac" starter, a starter designated as "Hansen" (a mixed strain starter) and a single strain starter designated as "M" were used. All three starters were obtained from the Provincial Dairy Branch Laboratory at Edmonton. Plate counts were determined of sterile skim milk soured by these starters and reported on the following page. Each count is the average of 5 replicate plates.

Total counts for skim milk soured by the three starters

Starter	25°C. T.G.E.M. Medium	25°C. veal infusion medium
Flavolac	950,000,000	620,000,000
Hansen	680,000,000	780,000,000
M	720,000,000	870,000,000
Average	780,000,000	760,000,000

It would seem that either medium (2) or medium (4) is satisfactory for maximum growth of the lactic streptococci. Consequently these media were used for the remainder of the study.

All counts given in this investigation are on a per millilitre basis.

(b) Methods of collection and examination of samples.

Forty-one sweet raw milks were collected and examined during the spring and summer seasons as follows:-

(1) Sweet raw milk samples were collected from three different plants designated A, B and C in Edmonton. A sterile wrapped pint bottle and a sterile wrapped thief were used to collect a composite sample representative of each of the milk cans any particular shipper sent to one of these plants. The date, plant at which the sample was collected and the shipper's number were recorded in order that a repeated analysis of that particular shipper's milk would not be made.

(2) At the laboratory methylene blue reduction tests were run on each sample collected (1).

(3) After starting the methylene blue reduction test the milk sample was plated on the veal infusion medium (medium (2)) and at the same time was plated on the T.G.E.M. agar (medium 4.).

(4) At the end of the incubation, a plate was selected with greater than 30 colonies and less than 300 and counted. A representative area of the plate containing 30 colonies was chosen and these colonies were picked into litmus milk and a brief description of each colony was recorded. Litmus milk cultures were incubated at 25°C. or 35°C. according to the temperature at which they were originally isolated. The 25°C. cultures were kept at that temperature for one week and the 35°C. cultures for 5 days before being discarded. In these intervals the cultures were observed periodically.

(5) The litmus milk cultures which were believed to be lactic streptococci were transferred to stock culture agar as recommended by Ayers and Johnson(4) for growth and long life of streptococci. These stock cultures were incubated at the same temperature at which they were originally isolated. When good growth was evident they were sealed with paraffin wax and stored at 40°F. for future reference. Smears of the litmus milk cultures were gram-stained and examined.

(6) Any of the litmus milk cultures that were believed to be lactic streptococci were transferred to litmus milk again and observed for growth at 45°C. If they grew at this temperature they were classed as fecal streptococci. If any fecal streptococcus cultures proteolyzed the curd of litmus milk or

liquefied gelatin they were included with the fecal streptococci.

Eighteen sweet raw milks were collected during the winter season from the same commercial plants that were the source of the sweet raw milks collected during the spring and summer seasons. These sweet milks were examined in the same manner as the spring and summer milks except that only the T.G.E.M. agar (medium 4) was used to examine the sweet milk. After the sweet milk was plated on this medium it was allowed to incubate at 25°C. until soured as indicated by a marked rise in titratable acidity. On souring it was plated on the veal infusion medium (medium 2) and at the end of the incubation period colonies were picked and examined as before.

Milks which were aseptically drawn from dairy cows on the University of Alberta stock farm were examined in precisely the same manner as the 18 raw winter milks were. No methylene blue reduction tests were run, however.

Ten pasteurized milks were collected from one of the commercial plants in Edmonton. Of these 10, six were obtained about 9 feet from the exit of the H.T.S.T. pasteurizer and 4 were collected from the bottler. As was done for the raw winter milks, analysis was carried out on both the sweet and soured pasteurized milks and the same techniques used.

Sour churning creams were tested for titratable acidity and the values were recorded. The veal infusion medium (medium 2) was used to plate these products and techniques of isolation and examination of cultures was the same as before.

(c) Incubation techniques.

The following temperature-time combinations were used when plating products examined in this investigation.

(1) Sweet milks:

- a. Spring and summer raw milks at both 25°C. for 4 days and 35°C. for 48 hours.
- b. Winter raw milks at 35°C. for 48 hours.
- c. Aseptically drawn milks at 35°C. for 48 hours.
- d. Pasteurized milks at 35°C. for 48 hours.

(2) Soured milks and creams.

- a. Winter raw milks at 25°C. for 4 days.
- b. Aseptically drawn milks at 25°C. for 4 days.
- c. Pasteurized milks at 25°C. for 4 days.
- d. Churning creams at 25°C. for 4 days.

(d) Calculation of the lactic or fecal streptococcus count.

In making this calculation an area of the plate containing 30 colonies was chosen and each colony was fished individually into litmus milk. The 30 colonies were assumed to be representative of the whole plate. The number of lactic streptococcus (or fecal streptococcus) cultures present in the 30 resulting pure cultures was determined. Then the percentage of the total number of colonies on the plate that were made up of these 30 colonies was computed. From the number of lactic or fecal streptococcus cultures and the percentage of the total number of colonies picked, the lactic (or fecal) streptococcus count was easily computed.

(e) Definitions

(1) Techniques.

When the 25°C. total count, the 25°C. lactic streptococcus count or the 25°C. veal infusion technique or just simply the 25°C. technique are mentioned it refers to all data obtained from counting and picking colonies from the veal infusion medium (medium 2) for a suitable dilution and for a particular sample after having been incubated at 25°C. for 4 days. Similarly when the 35°C. total count, the 35°C. lactic streptococcus count or the 35°C. - T.G.E.M. technique or the 35°C. technique are mentioned it refers to data of the same nature obtained from the T.G.E.M. medium (medium 4) after having been incubated at 35°C. for 48 hours. When the lactic streptococcus count is not prefixed with a temperature it means the higher lactic streptococcus count of two techniques. When only one technique is used the lactic streptococcus count is not prefixed with a temperature. What has been said of the lactic streptococcus counts holds for the fecal streptococcus counts also.

(2) The lactic streptococci (8,11) are those which form small lens to circular shaped subsurface colonies. Surface colonies are small, gray or milky, round and raised. These organisms reduce litmus milk completely with simultaneous acid production followed by the formation of a smooth, firm coagulum with or without whey formation. A narrow reoxidized band at the top of the coagulum gradually widens with age. The cells of the lactic streptococci are circular to egg-shaped and are less than one

micron in diameter. They occur in diplococcal forms or in chains of varying length.

(3) The fecal streptococci (8) have the same morphological characteristics as the lactic streptococci and give the same typical cultural reaction in litmus milk. Included with these fecal streptococci are those organisms which proteolyze the curd of litmus milk following the typical reaction in this medium and also liquefy gelatin. These proteolytic fecal streptococci (S. liquefaciens or S. zymogenes) are considered by Shattock(20) to be variants of S. fecalis, a typical non-proteolytic fecal streptococcus. The fecal streptococci can be differentiated from the lactic streptococci in that the fecal streptococci grow at 45°C. and the lactic streptococci do not.

Both the lactic streptococci and the fecal streptococci are gram positive.

(4) The acid formers (not lactic or fecal) give an acid reaction in litmus milk. Included with this group are acid formers which either coagulate, reduce or proteolyze litmus milk in a manner which is distinctly different from the manner in which the fecal streptococci or lactic streptococci do.

(5) The alkali formers cause no other change in litmus milk than to give it an alkaline reaction.

IV. ANALYSIS AND DISCUSSION OF RESULTS

A. Sweet Raw Milks

1. The lactic streptococcus count in relation to medium and incubation temperature.

In table 1 the lactic streptococcus counts, expressed as percentage of total plate counts, are averaged for both the spring and summer samples and are seen to vary little as between the two methods of isolation.

The average 25°C. lactic streptococcus count, for the spring and summer milks, was greater than the average 35°C. lactic streptococcus count.

The 41 spring and summer milks were the source of 1,230 isolations from the 35°C. - T.G.E.M. medium. Of these 115 were lactic streptococci. A similar number of cultures were isolated from the 25°C. - veal infusion medium and 114 were found to be lactic streptococci.

The 35°C. - T.G.E.M. medium gave the highest lactic streptococcus counts for 8 of the first 41 milks, while the other medium gave the highest lactic streptococcus counts in 11 of the milks. There were 18 of these 41 milks in which zero lactic streptococcus counts were obtained in both techniques. In addition there were 5 milks in which a lactic streptococcus count was obtained by the 35°C. technique and not by the other and 2 milks in which a lactic streptococcus count was obtained by the 25°C. technique and not by the other. Such

differences, however, were probably not significant.

The 35°C. technique gave 21 milks with lactic streptococcus counts above zero while the 25°C. technique gave 18. But the average lactic streptococcus count for the first 41 milks expressed as percentage of the average total count for these 41 milks, was higher for the 25°C. technique.

It is evident that for this purpose the two techniques of isolation are equal.

2. The lactic streptococcus count in relation to season

Since there is not a significant difference between the two techniques of isolation for the spring and summer raw milks, the higher lactic streptococcus counts and the 35°C. total counts were averaged for each season. These averages appear in table 2. The higher lactic streptococcus count will be the one used hereinafter in this report and will be designated simply as the lactic streptococcus count. It will be observed from table 1 that 3 of the 59 milks had lactic streptococcus counts of greater than 100,000. Two of these were spring milks and 1 was a winter milk. Excluding these 3 milks from the analysis, the remaining 56 milks were averaged in the same manner (table 2). Four spring milks, 2 summer milks and 1 winter milk had lactic streptococcus counts of greater than 20,000. Similarly the 7 were excluded from the analysis and the remaining 52 were averaged just as before and this also appears in table 2.

The winter milks were subjected to only one technique of analysis and that was the 35°C. - T.G.E.M. technique. Results are presented in table 1.

Including all of the 59 milks in the analysis appearing in table 2 it would seem that there is no great difference in numbers of lactic streptococci between the summer and winter milks. When the 3 milks, which have lactic streptococcus counts of greater than 100,000, are excluded from the analysis in table 2, there appears to be a marked difference in numbers between the summer and winter milks but not between the spring and summer milks. When the 7 milks, which have lactic streptococcus counts of greater than 20,000, are excluded from the analysis, the summer milks appear to have a higher incidence of lactic streptococci than the milks in the other two seasons. The data in tables 2 and 3 seem to indicate there is a lower incidence of lactic streptococci during the winter season but fails to prove conclusively a difference between the spring and summer seasons.

3. The lactic streptococcus count in relation to total isolations

The information obtained from tables 1, 2 and 3 indicate that the lactic streptococci are but a small percentage of the total bacterial flora occurring in milk.

Lactic streptococci were isolated from 27 of the 59 milks.

Of a total of 3,000 cultures isolated from the 59 milks, 248 (8.3%) were lactic streptococci.

While the lactic streptococci occur normally in raw milks, as will later be shown, it can be concluded here that the lactic streptococcus counts are on the average but a small percentage of the total plate count.

4. The lactic streptococcus count in relation to the plants from which the milks were collected

The three commercial plants which were the source of the 59 milks are given in table 1.

Of 29 milks collected from plant A, 38% had lactic streptococcus counts of zero, 17% had lactic streptococcus counts of greater than 20,000 and 45% were intermediate to these extremes. Of 25 milks collected from plant B, 72% had lactic streptococcus counts of zero, 4% had lactic streptococcus counts of greater than 20,000 and 24% were intermediate to these extremes. Only 5 milks were obtained from plant C, 3 which had lactic streptococcus counts of zero, 1 a lactic streptococcus count of greater than 20,000 while 1 was intermediate to these extremes.

The lactic streptococcus counts and the 35°C. total counts were averaged for the A and B plants. These averages are set down in table 4.

While the distribution of milks of high and low lactic streptococcus incidence and the data in table 4 give evidence that there is a higher concentration of lactic streptococci in the milks coming from plant A and a smaller concentration

in the milks from plant B, these differences may not be significant because of the few milks examined.

5. The lactic streptococcus count in relation to the 35°C. total count and the methylene blue reduction time

(a) Relation to the 35°C. total count

The relation between the lactic streptococcus counts and the 35°C. total counts for the 59 milks is shown in table 5. A glance at this table would seem to indicate that the increase in the lactic streptococcus count is more rapid than the increase in the 35°C. total count. For the majority of the milks, however, a very slight increase in the 35°C. total count gave a similar increase in the lactic streptococcus count. For 9 of the 59 milks, an evident increase in the 35°C. total count, gave a much greater increase in the lactic streptococcus count. Nine milks are not sufficient to prove conclusively that as the 35°C. total count increases, the lactic streptococcus count increases in a like manner.

(b) Relation to the methylene blue reduction time

A final classification of the 59 milks according to their methylene blue reduction times is arranged in table 6 along with lactic streptococcus counts for each class. It appears first that a fall in the methylene blue reduction time for any group of milks, results in an increase in numbers of lactic streptococci. When only 52 milks, with lactic streptococcus counts of less than 20,000, are contained in the analysis, a fall in the methylene blue reduction time reveals no noteworthy change in numbers of lactic streptococci. It should be

further observed that all the milks were of good quality as determined by their methylene blue reduction times. For good quality milks, then, nothing conclusive can be reached here that would intimate that a decrease in the methylene blue reduction time gives a resulting increase in incidence of lactic streptococci.

B. Aseptically Drawn Milks

In table 1 three aseptically drawn milks are shown. The first two are milks that were collected from the udder after the removal of the foremilk. They had a very low 35°C. total count and there was no evidence of any lactic streptococci comprising this flora. The last was a foremilk with a higher 35°C. total count and no lactic streptococci were isolated from it.

C. Pasteurized Milks

From the analysis carried out in table 7 it can be readily discerned that there was no evidence of any lactic streptococci comprising the bacterial flora of pasteurized milk.

D. Soured Milks and Creams

1. The lactic streptococcus count in relation to soured creams

The analysis of the 15 sour churning creams appears in tables 9 and 10. The average lactic streptococcus count was 340,000,000 or 79.1% of the 25°C. total count. Of a total of

450 cultures isolated from the 15 creams, 350 (77.8%) were lactic streptococci. All of the 15 creams contained considerable numbers of lactic streptococci. Since such a large percentage of the flora of these creams was composed of the lactic streptococci, it is safe to assert that these particular microorganisms are the ones responsible for the souring of raw churning creams in the Edmonton district.

2. The lactic streptococcus count in relation to soured raw milks

To determine the bacteria responsible for the souring of raw milks, 18 milks collected in the winter season were allowed to sour at 25°C. and duly examined and found to contain considerable numbers of lactic streptococci as shown in tables 9 and 10. The average lactic streptococcus count was 1,200,000,000 or 70.6% of the 25°C. total count. Of a total of 540 cultures isolated from the 18 milks, 349 (64.6%) were lactic streptococci.

Observing table 3 it will be noticed that the flora of the winter milks before souring has a low incidence of lactic streptococci and after souring, as shown in table 10, a high incidence. The increase in numbers of lactic streptococci in raw milk that is allowed to sour is illustrated more sharply by comparing tables 1 and 9. Here it can be seen that on souring the percentage of lactic streptococci increased from 8.3% to 70.6%. More than that 16 of the 18 milks on souring were positive for lactic streptococci. The two that did not contain any observable lactic streptococci (numbers 49 and 55 in table 1) had methylene blue reduction times of 10 hours

and were, therefore, good quality milks and in their production were not seeded with lactic streptococci. One other fact is apparent here and that is that 14 of the 18 milks prior to souring were negative for lactic streptococci but on souring 16 of the 18 were positive. It is evident then that the lactic streptococci are responsible for the souring of Edmonton's raw milks, and occur normally in Edmonton's raw milks.

3. The lactic streptococcus count in relation to soured aseptically drawn milks

Results for the aseptically drawn milks shown in table 1 indicate that these milks have low total counts provided the foremilk is not included with them. Table 9 reveals that on incubating these milks at 25°C. a much lower titratable acidity than the sour raw milks and the sour churning creams was attained. Furthermore while not indicated in table 9 such milks took a considerably longer period to arrive at this acidity than other milks. On differentiating the kinds of bacteria in these incubated milks, no lactic streptococci were obtained. This suggests that the interior of the udder is not a suitable habitat for the lactic streptococci.

4. The lactic streptococcus count in relation to soured pasteurized milk

The pasteurized milks collected from plant B, were allowed to sour at 25°C. and then examined. Milks 1 to 6 in table 7 were obtained about 9 feet from the exit of the H.T.S.T. pasteurizer at the beginning of the day's operations. The last 4 were collected from the bottler during the day's run. While all of

the 10 sweet pasteurized milks had lactic streptococcus counts of zero, the 4 soured milks that originally came from the bottler, shown in table 9, had considerable numbers of lactic streptococci. One of the milks (number 6), from the H.T.S.T. pasteurizer, had a large lactic streptococcus count.

The average lactic streptococcus count for the 10 soured pasteurized milks was 550,000,000 or 39.3% of the 25°C. total count. The average lactic streptococcus count for the 5 soured pasteurized milks containing observable lactic streptococci was 1,100,000,000 or 84.6% of the 25°C. total count. Of a total of 150 cultures isolated from the 5 soured milks positive for lactic streptococci (table 11), 107 (71.3%) were lactic streptococci.

The five soured pasteurized milks that contained no observable lactic streptococci (numbers 1 to 5) on coming off the H.T.S.T. pasteurizer, gave no evidence of seeding with lactic streptococci. Number 6 milk on coming off the pasteurizer was probably seeded with lactic streptococci in the pipeline. The 4 milks collected from the bottler must have had small numbers of lactic streptococci introduced into them in the process of going through pipelines and collection tanks.

It can be concluded that lactic streptococci are killed on pasteurizing raw milks. However, these same pasteurized milks can easily be seeded from non-sterile surfaces with small numbers of lactic streptococci which, if conditions are favorable, can sour pasteurized milk.

E. The Fecal Streptococci in Milks and Creams

The highest fecal streptococcus counts and the 35°C. total counts in table 1 were averaged for each season. Milk 5, however, was excluded since it had an abnormally high fecal streptococcus count. The averages for these 58 milks are shown in table 12. The data in tables 3 and 12 do not seem to indicate that there is a great difference in numbers of fecal streptococci between the spring and summer milks, but there seems to be a lower incidence of these microorganisms in the winter milks. Comparing tables 2 and 12 it can be easily seen that the fecal streptococci in the milks for the 3 different seasons are of much lower incidence than the lactic streptococci.

The fecal streptococci make up a small percentage of the total bacterial flora occurring in raw milks according to the information coming from tables 1, 3 and 12. Fecal streptococci were isolated from only 9 of the 59 milks. Of a total of 3,000 cultures isolated from these 59 milks, 55(1.8%) were fecal streptococci. It is not normal for milks to have large numbers of fecal streptococci .

Fecal streptococci were not isolated from sweet or soured aseptically drawn milks, sweet or soured pasteurized milks or sour churning creams but were isolated in large numbers from some of the soured winter raw milks. Observing table 3 it will be noticed that the fecal streptococcus incidence of the winter milks before souring was low and after souring, as shown in table 10, was higher. Comparing tables 1 and 9 it can be seen that in soured winter milks the percentage of fecal streptococci

increased from 0.1% to 5.3%. This increase in fecal streptococci in the sour raw milks was not as great as the increase in lactic streptococci.

It can be concluded at this point that the fecal streptococci constitute a much smaller percentage of the total plate count in both sweet and soured milks and are not usually responsible for the souring of Edmonton's milks and creams.

F. Other Bacteria in Milks and Creams

The acid formers (not lactic or fecal), alkali formers and those bacteria which caused no observable change in litmus milk, as shown in table 3, constituted a greater percentage of the total bacterial flora of sweet raw milks than the lactic or fecal streptococci did. Of the 3 groups, the acid formers (not lactic or fecal) constituted the greatest proportion. The same acid formers as shown in table 8 constituted a greater percentage of the bacterial flora of sweet pasteurized milks.

In the sour milks and creams shown in tables 10 and 11, those products which had a preponderance of lactic streptococci, had a lower incidence of other acid formers. Such milks as the 5 soured pasteurized milks which did not contain any observable lactic streptococci and the soured aseptically drawn milks had a larger proportion of acid formers (not lactic or fecal).

Alkali formers were present in the lowest proportions in both the sweet and sour milks.

It can be concluded that milks not seeded with lactic streptococci may be soured by acid formers (not lactic or fecal).

G. Species Identification of 105 Cultures

Harrison(12) chose at random 105 cultures that were isolated during this investigation and identified them as *S. lactis*, *S. cremoris*, *S. fecalis* or *S. liquefaciens*. In every case but one his species identification confirmed the original lactic and fecal streptococcus groupings.

CONCLUSIONS

In studying the occurrence and distribution of the lactic streptococci in Edmonton's milks and creams, the following conclusions were arrived at:-

1. The average lactic streptococcus count for the sweet raw milks was lower in the winter than in the summer season.
2. While the lactic streptococci occur normally in Edmonton's sweet raw milks they are on the average but a small percentage of the total bacterial flora of these milks.
3. The lactic streptococci are usually responsible for souring of sweet raw milks, churning creams and some pasteurized milks in the Edmonton area and when they are not - or only partly responsible - the fecal streptococci or acid formers (not lactic or fecal) are responsible.
4. The fecal streptococci are present in insignificant numbers in Edmonton's sweet raw milks and are not usually responsible for their souring.

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TABLE 1

Total, lactic streptococcus and fecal streptococcus counts of the spring, summer, winter and aseptically drawn milks

Season	Milk Number	Total counts		Lactic streptococcus counts		Fecal streptococcus counts		Plant	Methylene blue reduction time
		35°C.	25°C.	35°C.	25°C.	35°C.	25°C.		
Spring milks	1	16,000	20,000	0	0	0	0	A	8
	2	52,000	19,000	0	0	0	0	B	10
	3	2,000	3,000	0	0	0	0	A	10
	4	77,000	57,000	34,000	32,000	0	0	A	5½
	5	250,000	200,000	8,000	0	83,000	80,000	B	6½
	6	11,000	14,000	0	0	0	0	C	8½
	7	190,000	110,000	39,000	39,000	0	0	A	7
	8	14,000	19,000	500	3,000	0	0	B	8
	9	7,000	11,000	500	400	0	0	B	8
	10	38,000	44,000	0	0	0	0	B	10
	11	160,000	150,000	5,000	0	0	0	A	5
	12	4,000	4,000	200	1,000	0	0	A	9½
	13	10,000	14,000	0	0	0	0	B	10
	14	53,000	58,000	0	0	0	0	B	6
	15	170,000	100,000	6,000	0	0	0	C	7½
	16	160,000	290,000	89,000	160,000	0	0	C	8
	17	39,000	42,000	4,000	9,000	8,000	14,000	A	10
	18	200,000	290,000	91,000	130,000	0	0	A	5
	19	45,000	30,000	0	0	0	0	C	10
	20	3,000	4,000	0	0	0	0	C	10
	Average	75,000	74,000	14,000	19,000	5,000	5,000		8
	% of average total count			18.7%	25.7%	6.7%	6.8%		
Summer milks	21	21,000	16,000	0	0	0	0	A	10
	22	70,000	76,000	0	0	0	0	A	6½
	23	20,000	19,000	0	2,000	0	0	A	9
	24	26,000	51,000	9,000	0	0	0	A	9
	25	120,000	130,000	4,000	4,000	16,000	4,000	A	8½
	26	47,000	49,000	14,000	7,000	0	0	A	10
	27	44,000	42,000	2,000	3,000	2,000	0	A	8
	28	50,000	82,000	22,000	33,000	4,000	8,000	B	8½
	29	14,000	16,000	0	0	0	0	B	10
	30	15,000	32,000	3,000	3,000	0	0	B	9½
	31	46,000	60,000	5,000	0	3,000	8,000	B	8½
	32	30,000	35,000	0	0	0	0	B	10
	33	220,000	300,000	0	0	0	0	B	5½
	34	19,000	16,000	0	0	0	0	B	9
	35	18,000	26,000	4,000	5,000	3,000	2,000	B	8½
	36	490,000	440,000	16,000	29,000	0	0	A	5
	37	120,000	230,000	0	15,000	0	0	A	8
	38	2,000	4,000	0	0	0	0	B	10
	39	90,000	120,000	0	0	0	0	B	10
	40	5,000	4,000	0	0	0	0	A	10
	41	60,000	180,000	10,000	6,000	4,000	0	A	8
	Average	73,000	92,000	4,000	5,000	2,000	1,000		8½
	% of average total count			5.5%	5.5%	2.7%	1.1%		
Spring and summer milks	Average	74,000	83,000	9,000	12,000	4,000	3,000		
	% of average count			12.2%	14.5%	5.4%	3.6%		
Winter milks	42	100,000		7,000		0		A	10
	43	54,000		9,000		0		A	10
	44	61,000		0		0		A	10
	45	48,000		0		0		A	10
	46	67,000		4,000		0		A	6½
	47	150,000		0		0		A	10
	48	190,000		0		0		B	5
	49	5,000		0		0		B	10
	50	220,000		0		0		B	5
	51	11,000		0		0		B	8½
	52	19,000		0		0		B	7
	53	160,000		0		0		B	10
	54	12,000		0		0		A	8
	55	50,000		0		0		A	10
	56	300,000		0		0		A	9½
	57	490,000		160,000		0		A	5
	58	60,000		0		0		B	8
	59	96,000		0		3,000		B	8
	Average	120,000		10,000		170			8
	% of average total count			8.3%		0.1%			
Aseptically drawn milks	A	300		0		0			
	B	250		0		0			
	C	88,000		0		0			

Summer milks	25	120,000	130,000	4,000	4,000	16,000	4,000	A	8½
	26	47,000	49,000	14,000	7,000	0	0	A	10
	27	44,000	42,000	2,000	3,000	2,000	0	A	8
	28	50,000	82,000	22,000	33,000	4,000	8,000	B	8½
	29	14,000	16,000	0	0	0	0	B	10
	30	15,000	32,000	3,000	3,000	0	0	B	9½
	31	46,000	60,000	5,000	0	3,000	8,000	B	8½
	32	30,000	35,000	0	0	0	0	B	10
	33	220,000	300,000	0	0	0	0	B	5½
	34	19,000	16,000	0	0	0	0	B	9
	35	18,000	26,000	4,000	5,000	3,000	2,000	B	8½
	36	490,000	440,000	16,000	29,000	0	0	A	5
	37	120,000	230,000	0	15,000	0	0	A	8
	38	2,000	4,000	0	0	0	0	B	10
	39	90,000	120,000	0	0	0	0	B	10
	40	5.000	4.000	0	0	0	0	A	10

TABLE 2

Average of the lactic streptococcus and 35°C. total counts for the three seasons

Number of milks	Description of count	Spring	Summer	Winter	Average
59	Average lactic streptococcus count	20,000	6,000	10,000	12,000
	Average 35°C. total count	75,000	73,000	120,000	89,000
	% lactic streptococci	26.9%	8.2%	8.3%	14.5%
56	Average lactic streptococcus count	6,000	6,000	1,000	4,000
	Average 35°C. total count	63,000	73,000	94,000	77,000
	% lactic streptococci	9.5%	8.2%	1.1%	6.3%
52	Average lactic streptococcus count	2,000	4,000	1,000	2,000
	Average 35°C. total count	55,000	52,000	94,000	67,000
	% lactic streptococci	3.6%	7.7%	1.1%	4.1%

TABLE 3

Number of cultures isolated in each of three seasons from the raw milks

Type of culture	Spring milks	Summer milks	Winter milks
Acid formers (not lactic or fecal)	731	745	356
Lactic streptococci	137	82	19
Fecal streptococci	38	25	1
Alkali formers	74	151	31
No observable change	220	257	133
Total	1,200	1,260	540
Lactic streptococci as % of total	11.4%	6.5%	3.5%
Fecal streptococci as % of total	3.2%	2.0%	0.2%

TABLE 4

The average of the lactic streptococcus and 35°C. total counts for the milks coming from plants A and B

Plant A 29 milks	Average lactic streptococcus count	18,000
	Average 35°C. total count	100,000
	% lactic streptococci	18.0%
Plant B 25 milks	Average lactic streptococcus count	2,000
	Average 35°C. total count	69,000
	% lactic streptococci	2.9%

TABLE 5

Total counts of 59 milks classed according to the lactic streptococcus count

Lactic streptococcus count range	Number of samples in each class	Average lactic streptococcus count in each class	Average 35°C. total count for each class
0	32	0	65,000
0⊲ – ⊴10,000	18	5,000	67,000
10,000⊲ – ⊴20,000	2	15,000	84,000
⊳20,000	7	84,000	240,000

TABLE 6

Lactic streptococcus counts of the milks classed according to their methylene blue reduction time

Number of milks	Methylene blue reduction range	Number of samples	Average methylene blue reduction time	Average lactic streptococcus count
59	⊳5 – ⊲7½	15	6	28,000
	⊳8 – ⊲8½	16	8	15,000
	⊳9 – ⊲10	28	9	2,000
52	⊳5 – ⊲7½	10	6	2,000
	⊳8 – ⊲8½	14	8	3,000
	⊳9 – ⊲10	28	9	2,000

TABLE 7

Total and lactic streptococcus counts of the pasteurized milks

Milk number	35°C. total count	Lactic streptococcus count	Fecal streptococcus count	Plant
1	24,000	0	0	B
2	23,000	0	0	B
3	26,000	0	0	B
4	38,000	0	0	B
5	88,000	0	0	B
6	66,000	0	0	B
7	53,000	0	0	B
8	45,000	0	0	B
9	30,000	0	0	B
10	39,000	0	0	B
Average	43,000	0	0	

TABLE 8

Cultures isolated from the pasteurized milks

Type of culture		Number of cultures
Acid formers (not lactic or fecal)		230
Alkali formers		48
No change		22
	Total	300

TABLE 9

Total and lactic streptococcus counts of soured

milks and creams

Type of product	Milk number	Titratable acidity	25°C. total count	Lactic streptococcus count	Fecal streptococcus count
Sour creams	1	0.27%	170,000,000	160,000,000	0
	2	0.46	350,000,000	210,000,000	0
	3	0.54	660,000,000	590,000,000	0
	4	0.49	360,000,000	320,000,000	0
	5	0.30	300,000,000	180,000,000	0
	6	0.40	106,000,000	55,000,000	0
	7	0.36	340,000,000	330,000,000	0
	8	0.59	310,000,000	270,000,000	0
	9	0.25	470,000,000	240,000,000	0
	10	0.64	410,000,000	230,000,000	0
	11	0.47	1,300,000,000	1,100,000,000	0
	12	0.55	1,000,000,000	900,000,000	0
	13	0.55	750,000,000	730,000,000	0
	14	0.55	500,000,000	320,000,000	0
	15	0.63	1,200,000,000	1,200,000,000	0
	Average	0.47%	430,000,000	340,000,000	0
	% of average total count			79.1%	0%
Soured winter milks	1	0.32%	980,000,000	820,000,000	0
	2	0.57	610,000,000	510,000,000	0
	3	?	720,000,000	650,000,000	0
	4	?	440,000,000	180,000,000	40,000,000
	5	0.36	760,000,000	480,000,000	0
	6	0.72	4,300,000,000	4,300,000,000	0
	7	0.61	1,900,000,000	1,300,000,000	140,000,000
	8	0.37	1,900,000,000	0	0
	9	0.59	2,200,000,000	370,000,000	510,000,000
	10	0.58	1,100,000,000	730,000,000	0
	11	0.81	4,200,000,000	4,200,000,000	0
	12	0.73	1,800,000,000	1,600,000,000	180,000,000
	13	0.79	2,400,000,000	1,100,000,000	80,000,000
	14	0.59	820,000,000	0	0
	15	0.63	2,300,000,000	2,100,000,000	0
	16	0.57	780,000,000	550,000,000	50,000,000
	17	0.65	1,800,000,000	1,300,000,000	300,000,000
	18	0.72	2,400,000,000	2,100,000,000	320,000,000
	Average	0.60%	1,700,000,000	1,200,000,000	90,000,000
	% of average total count			70.6%	5.3%
Soured aseptically drawn milks	A	0.28%	560,000,000	0	0
	B	0.33	700,000,000	0	0
	C	0.35	140,000,000	0	0
	Average	0.32%	470,000,000	0	0
	% of average total count			0%	0%
Soured pasteurized milks	1	0.70%	1,900,000,000	0	0
	2	0.57	200,000,000	0	0
	3	0.75	1,600,000,000	0	0
	4	0.70	2,100,000,000	0	0
	5	0.47	1,600,000,000	0	0
	6	0.62	980,000,000	880,000,000	0
	7	0.32	330,000,000	22,000,000	0
	8	0.80	3,100,000,000	3,100,000,000	0
	9	0.78	580,000,000	460,000,000	0
	10	0.75	1,300,000,000	1,000,000,000	0
	Average	0.65%	1,400,000,000	550,000,000	0
	% of average total count			39.3%	0%
Pasteurized milks 6 - 10	Average	0.65%	1,300,000,000	1,100,000,000	0
	% of total count			84.6%	0%

winter	9	0.59	2,200,000,000	370,000,000	510,000,000
milks	10	0.58	1,100,000,000	730,000,000	0
	11	0.81	4,200,000,000	4,200,000,000	0
	12	0.73	1,800,000,000	1,600,000,000	180,000,000
	13	0.79	2,400,000,000	1,100,000,000	80,000,000
	14	0.59	820,000,000	0	0
	15	0.63	2,300,000,000	2,100,000,000	0
	16	0.57	780,000,000	550,000,000	50,000,000
	17	0.65	1,800,000,000	1,300,000,000	300,000,000
	18	0.72	2,400,000,000	2,100,000,000	320,000,000
	Average	0.60%	1,700,000,000	1,200,000,000	90,000,000
	% of average total count			70.6%	5.3%
Soured	A	0.28%	560,000,000	0	0
aseptically	B	0.33	700,000,000	0	0
drawn milks	C	0.35	140,000,000	0	0
	Average	0.32%	470,000,000	0	0
	% of average total count			0%	0%
	1	0.70%	1,900,000,000	0	0
	2	0.57	200,000,000	0	0
	3	0.75	1,600,000,000	0	0
Soured	4	0.70	2,100,000,000	0	0

TABLE 10

Number of cultures isolated from soured milks and sour churning cream

Type of culture	Sour creams	Soured winter milks	Soured aseptically drawn milk
Acid formers (not lactic or fecal)	74	150	48
Alkali formers	0	5	0
Lactic streptococci	350	349	0
Fecal streptococci	0	27	0
No observable change	26	9	32
Total	450	540	80
Lactic streptococci as % of total	77.8%	64.6%	0%
Fecal streptococci as % of total	0%	5.0%	0%

TABLE 11

Number of cultures isolated from soured pasteurized milks with and without observable lactic streptococci

Type of culture	Pasteurized milks 1 to 5	Pasteurized milks 6 to 10
Acid formers (not lactic or fecal)	145	29
Alkali formers	0	6
Lactic streptococci	0	107
No observable change	5	8
Total	150	150
Lactic streptococci as % of total	0%	71.3%

TABLE 12

Average of the fecal streptococcus and 35°C. total counts for the three seasons

Description of count	Spring	Summer	Winter
Average fecal streptococcus count	700	2,000	170
Average 35°C. total count	66,000	73,000	120,000
% fecal streptococci	1.1%	2.7%	0.1%